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Research Article

Effect of *Fusarium* sp. On Paddy Straw Digestibility and Biogas Production

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Abstract: Chopped and moist paddy straw was pretreated with *Fusarium* sp. to enhance its digestibility and biogas production. The potential of microbial pretreatment of paddy straw was investigated at regular interval of 5, 10, 15 and 20 days by determining the change in Chemical composition of paddy straw like cellulose, hemicellulose, lignin and silica contents. The pretreated straw was used for biogas production in 2l capacity biogas digesters. Results indicate that the cellulose, hemicellulose, lignin and silica contents decreased by 17.2%, 3.4%, 27.1% & 16.5% respectively. Biogas production also increased by 53.8% in 10 days pretreated samples. The significantly higher reduction of silica along with lignin content in the pretreated straw indicates that removal of silica by *Fusarium* sp. might be more responsible for increasing paddy straw digestibility and biogas production.

Keywords: Biodegradation, Biogas production, Lignin, Paddy straw, *Fusarium* sp., Silica.

1. Introduction

The lignocellulosic biomasses are found to be abundant on earth, which is not fruitfully utilized. This can be converted to biogas *via* anaerobic digestion (Kashyap *et al.,* 2003). Anaerobic digestion is a biological process in which biodegradable organic material decomposes in the absence of oxygen to produce biogas which is a mixture of CH_4 (55-75%), CO_2 (25-45%), H_2 (0-3%), N_2 (1-5%), CO (0-0.3%), H_2S (0.1-0.5%), O_2 and water vapors (traces) (Pauss *et al.,* 1987). The organic matter can be degraded by the sequential action of hydrolytic, acetogenic and methanogenic bacteria to produce biogas.

Paddy straw is one of the most abundant cellulosic wastes on the earth. In India, total annual production of rice is estimated to be 136.5 million tons (Anonymous, 2010). About 1-1.5kg of straw is produced from every kilogram of the grain harvested (Maiorella, 1985) and thus, 136.5-150 million tons of paddy straw is estimated to be produced annually. In India, approximately 85-95 million tons of paddy straws are disposed of by burning. One ton of paddy straw burning releases 3kg particulate matter, 60kg CO, 1460kg CO_2, 199kg ash and 2kg SO_2 (Jenkins and Bhatnagar, 2003). Burning of paddy straw causes lung and respiratory diseases (Wang and Christopher, 2003). Repeated burning of paddy straw also results in soil erosion. Paddy straw consists of cellulose (35-40%), hemicellulose (20-24%), lignin (8-12%), ash (14-16%) and extractives (10-12%). Although, paddy straw has high cellulose content but the lignin complex and silica incrustation shield the microbial action for biogas production.

Therefore, the paddy straw needs to be pretreated in order to enable cellulose to be more accessible to the microbial/enzymatic attack. Many physical (mechanical and non-mechanical), chemical (alkaline hydrolysis, acid hydrolysis, oxidative delignification and solvent extraction), physicochemical (ammonia fibre explosion, CO_2 and steam explosion) and biological pretreatments (lignocellulolytic microorganisms and the enzymes) have been proposed in the recent years (Saratale *et al.,* 2008; Hendriks and Zeeman, 2009). However, the physical and chemical pretreatments require high energy, corrosion resistant and high-pressure reactors, which increase the need for sophisticated equipment

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and cost of pretreatment. Furthermore, the chemical pretreatments can be detrimental to the methanogens apart from generating acidic or alkaline water, which needs predisposal treatment to ensure environmental safety (Keller *et al*., 1987).

Thus, an alternative approach is microbial pretreatment which employs the use of microorganisms especially fungi to increase digestibility of paddy straw. In biological pretreatment processes, microorganisms such as brown, white and soft rot fungi are used to degrade lignin and hemicellulose in waste materials (Schurz and Ghose, 1978). Advantages of biological pretreatment include cost-effectiveness, low energy requirement and mild environmental conditions (Saratale *et al*., 2008). Most of the white-rot fungi degrade lignin and cellulose simultaneously. A selective white-rot fungus, *Ceriporiopsis subvermispora* is known to selectively degrade lignin in softwood and hardwood (Okano *et al*., 2005).

Mandhulika *et al*., (1993) isolated various lignocellulolytic fungi and reported that *Aspergillus* sp., *Paecilomyces* sp. and *Sporotrichum* sp. were most efficient lignocellulose degraders. No doubt, reports are available for biological pretreatment of paddy straw; however, no work has been reported on the effect of pretreatment on biogas production. Therefore, the present study was undertaken to optimize the conditions for biological pretreatment of paddy straw by *Fusarium* sp. and to study the implication of enhanced paddy straw digestibility on biogas production.

2. Materials and Methods

2.1 Procurement of the materials

Fresh paddy straw was procured from the research field of Punjab Agricultural University, Ludhiana after harvesting of the crop in the month of October and November. The paddy straw was chopped to 3-4cm size with a Toka machine and was stored in polythene bags at room temperature. All the chemicals used for media, solutions preparation and proximate analysis were of analytical grade and were purchased from HiMedia, Loba Chemie and Sd fine chemicals Pvt. Ltd. Cattle dung was procured from Dairy farm, GADVASU (Guru Angad Dev Veterinary and Animal Science University), Ludhiana. The digested cattle dung slurry was procured from a working biogas plant in the bogus field laboratory of School of Energy Studies for Agriculture, PAU, Ludhiana and was used as inoculum for biogas production.

2.2 Pretreatment of paddy straw

For the preparation of inoculum of *Fusarium* sp., wheat grains were washed and boiled for 20-30 minutes. The excess water was drained off. The grains were then mixed with 2% gypsum ($CaSO_4$) and 4% $CaCO_3$. The grains were dispensed into empty glucose bottles (250g/bottle). The bottles were plugged and autoclaved for 90 minutes. After cooling, the bottles were inoculated with 7-8 days old culture of *Fusarium* sp. and incubated at 30 ± 2^0C. The mycelium impregnated grains were used to inoculate paddy straw. Chopped paddy straw was soaked in water overnight. The excess water was drained off, so as to have approximately 70% moisture content. It was mixed with inoculum at the rate of 10% w/w ratio (i.e. 25g inoculum /250g PS). After proper mixing, paddy straw was filled in polythene bags and incubated at 30 ± 2^0C for different durations of time i.e. 5 days, 10 days, 15 days and 20 days, respectively. After the completion of required incubation, one set of treated paddy straw was removed and was used to determine the change in paddy straw composition i.e. cellulose, hemicellulose, lignin and silica content as well as biogas production. The same procedure was followed after 10 days, 15 days and 20 days.

Biogas production experiments were carried out in glass biogas digesters of 2 litre capacity following single phase digestion and biogas produced was measured by water displacement method for a period of 35 days. The 250gm pretreated paddy straw was mixed with 250ml of the digested cattle dung slurry and 100gm cattle dung. The mixture was fed to the biogas digesters which were incubated at 37 ± 2^0C. A control was also run where untreated paddy straw was used instead of treated paddy straw. The experiment was conducted in triplicate.

2.3 Analytical procedures and statistical analysis

For the determination of total solids, volatile solids, ash, cellulose, hemicellulose, lignin and silica content of paddy straw, Standard methods (AOAC, 2000) were followed. The Standard error (SE at the 5% level) and Critical difference (CD at 5% level) was calculated for triplicate data.

3. Results and Discussion

In the present study, the chopped and soaked paddy straw was pretreated with *Fusarium* sp. and its effect on change in chemical composition of paddy straw was determined. The correlation between change in various contents of paddy straw and biogas production was developed. The results of various experiments conducted are discussed under different subheads:

3.1 Effect of *Fusarium* sp. on paddy straw degradation

Chopped and soaked paddy straw was pretreated with a lignocellulolytic fungus, *Fusarium* sp. and its effect on paddy straw digestibility was determined. The change in chemical (TS, VS, Ash and TOC content) and proximate (Cellulose, Hemicellulose, Lignin and Silica content) composition of paddy straw with enhanced biogas production was taken as criteria for paddy straw digestibility. Results from Table 1 indicate that there was a smooth and a significant decrease in TS and VS with an increase in the incubation period. The total solids decreased from 25.2% (in control) to 21.0% in 20 days treatment indicating a decrease of 16.7%. Volatile solids also decreased from 81.4% (in control) to 75.1% indicating a decrease of 7.7%. The cellulose content kept on decreasing with the increasing incubation period, with the maximum reduction of 17.2% after 20 days pretreatment. Initially, a decreasing trend of hemicellulose content was observed for a period of 15 days, however, further increase in the pretreatment period led to increases in hemicellulose content. The initial decrease in hemicellulose content might be the result of breakdown or hydrolysis of hemicellulose into fermentable sugars (Jalc *et al*., 1998). This observation clearly indicates that the fungus has active hemicellulases during the early phase of its growth cycle. A maximum of 87.4mg total sugars/g PS was found in 20 days treated paddy straw. Lignin content decreased with the increase of incubation period with maximum reduction of 27.1% in 20 days treated sample. Decrease in silica was smooth and significant with a maximum removal of 16.5% after 20 days. These observations clearly indicate that *Fusarium* sp. is lignocellulolytic fungus. However, drastic decrease in silica and lignin contents indicates that *Fusarium* sp. could be a potential organism for silica and lignin degradation/utilization.

Zafar *et al*., (1980) also observed that cellulose content of rice straw treated with *Pleurotus sajor-caju* decreased from 45.0% to 17.8%. Mehta *et al*., (1990) also, found a decrease in silica and increase in ash content in spent rice straw used for mushroom cultivation of *Pleurotus florida*. Shi *et al*., (2009) pretreated the cotton stalks with *Phanerochaete chrysosporium* and found a significant decrease in lignin i.e. 19.38% and 35.33% for submerged and solid-state cultivation respectively.

3.2 Effect of *Fusarium* sp. pretreatment on biogas production

Results from Table 2 indicate that biogas production increased with increasing pretreatment period till 20 days. A maximum of 53.8% increase in biogas production was observed in 10 days pretreated straw. The increase in biogas production might be due to the increase in digestibility of paddy straw by a decrease in silica and lignin content and breakage of bonds between cellulose, hemicellulose and lignin content. However, less biogas production after 15 days can be correlated with the decrease in cellulose content of paddy straw with increasing incubation period. The lignin hinders the paddy straw digestibility and cellulose is the preferred substrate for biogas production.

Two of the most important factors to be considered when any lignocellulosic biomass is subjected to methane fermentation is rate of degradation and extent of degradation. High biodegradability means not only more methane generated per unit feed mass but also that fewer residue results for subsequent disposal. Both these factors are functions of the intrinsic properties of the lignocellulosic material itself and the microorganisms involved (Bisaria and Ghose, 1981).

Table 1. Change in chemical and proximate composition of paddy straw pretreated with *Fusarium* sp.

	Pretreatment period (days)					CD at
Composition	**Control#**	**5 (% change)**	**10 (% change)**	**15 (% change)**	**20 (% change)**	**5 %**
Total solids (TS%)	25.2±0.13	24.9±0.15(1.2↓)	22.1±0.11(12.3↓)	21.5±0.16(14.7↓)	21.0±0.09(16.7↓)	0.20
Volatile solids (VS%)	81.4±0.07	78.5±0.10(3.6↓)	76.9±0.05(5.5↓)	75.8±0.07(6.9↓)	75.1±0.06(7.7↓)	0.13
Ash (%)	18.6±0.06	21.5±0.05(15.6↑)	23.1±0.07(24.2↑)	24.2±0.05(30.1↑)	24.9±0.07(33.8↑)	0.18
Total organic carbon (%)	45.2±0.14	43.1±0.10(4.6↓)	42.7±0.20(5.5↓)	42.1±0.17(6.8↓)	41.7±0.11(7.7↓)	0.15
Cellulose (%)	39.0±0.12	36.6±0.11(6.2↓)	32.8±0.13(15.9↓)	32.6±0.21(16.4↓)	32.3±0.23(17.2↓)	0.16
Hemicellulose (%)	26.8±0.22	26.0±0.15(2.9↓)	25.2±0.13(5.9↓)	25.0±0.22(6.7↓)	25.9±0.21(3.4↓)	0.14
Lignin (%)	7.0±0.12	6.4±0.06(8.6↓)	5.8±0.08(17.1↓)	5.4±0.14(22.8↓)	5.1±0.09 (27.1↓)	0.16
Silica (%)	12.0±0.06	11.4±0.07(1.3↓)	10.8±0.06(12.2↓)	10.5±0.08(14.6↓)	10.1±0.09(16.5↓)	0.18
Total sugar (mg/g PS)	43.0±0.07	62.2±0.05(42.1↑)	76.3±0.07(74.8↑)	82.1±0.08(87.8↑)	87.4±0.06(99.4↑)	0.13
TS (g/kg PS)	252.2±0.17	249.1±0.13(7.1↓)	221.3±0.14(12.3↓)	215.2±0.16(14.6↓)	210.4±0.12(16.7↓)	0.33
VS (g/kg PS)	205.7±0.11	195.5±0.13(4.9↓)	170.1±0.18(17.5↓)	162.9±0.16(20.8↓)	157.5±0.21(23.4↓)	0.33

#Control: Untreated paddy straw; PS: Paddy straw; CD: critical difference at 5% level; ± Values indicate % Standard error for triplicate data; ↓: decrease; ↑: increase

Table 2. Biogas production profile of *Fusarium* sp. pretreated paddy straw.

Parameters	Pretreatment period (days)				
	Control#	**5**	**10**	**15**	**20**
Biogas (l/250g PS)	30.1±9.4	35.5±8.9	46.3±11.6	37.1±12.2	31.4±13.3
Biogas (l/kg PS)	120.4±12.4	142.0±15.5	185.2±23.5	148.4±20.8	125.6±22.2
Biogas (l/kg TS)*	477.4±20.5	570.1±13.3	837.3±22.8	689.3±19.5	596.9±19.2
Biogas (l/kg VS)*	585.3±14.6	726.3±17.3	1088.7±19.8	910.9±24.6	797.5±21.8
% age change from control	0.0	17.9 (↑)	53.8 (↑)	23.3 (↑)	4.3 (↑)

#Control: Untreated paddy straw; PS: Paddy straw; *The values of biogas l/kg TS and biogas l/kg VS taken from Table 1; Average of the triplicate data composition of paddy straw mixture : 250g paddy straw + 250gm digested cattle dung slurry + 100g cattle dung; Biogas digester : 2 litre capacity; incubation temperature : 37 ± 2^{0}C; Incubation period: 35 days; The amount of biogas produced from cattle dung & slurry was deducted from the amount of biogas produced from paddy straw.

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